DAKSH GROUP

Presents

MANOUVRE – SERIES BOOK

PATHOLOGICAL TESTS PROCEDURES

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This is a compilation of all pathological tests in use till now in laboratories.

It does not propose any new theory, all these are a summary of all present pathological procedures followed in laboratories.

CSF Examination

CSF can be obtained through Lumbar Puncture. Normal CSF is clear and colorless like water.

1) **Yellowness of CSF is pathological. Could be either due to hemorrhage, jaundice or excess protein.**
2) **Turbidity may be due to presence if WBC, either due to injection or sub-arachnoid hemorrhage. Turbidity doesn't clear during standing, due to micro-organisms.**
3) **Presence of blood may be due to injury to vessel by the needle or sub-arachnoid hemorrhage.**
4) **Staining of CSF might show bacteria. Cell counts are performed in Counting chamber, must be done in fresh sample of CSF.**
5) **Bacterial Meningitis is associated with Viral Meningitis and Tubercular Meningitis. It could be Lymphocytic or Mixed type(Pleocytosis)**
6) **Cytological examination may reveal Malignant cells in patients with Secondary neoplastic invasion of Meninges from Lymphoma or Carcinoma.**
7) **Normal CSF does not contain albumin or globulin. It might be present in cases of Neurological disease like Multiple Sclerosis.**
8) **Normal CSF glucose concentration is half than that of blood glucose concentration. It decreases in Purulent Tubercular or Fungal Meningitis.**

Blood Examination

1) **Malaria – Low white blood cell count**
   (4×10^9/ litre)
   Thrombocytopenia
   Increased number of Atypical Lymphocytes
   Species of Malaria should be identified.
   Plasmodium Vivax, Plasmodium Falciparum, Plasmodium Ovale, Plasmodium Malariae
   Absolute number of parasites can be estimated by counting parasites against the patient's WBC
   Malaria pigment seen within the cytoplasm of Monocyte and Neutrophils, and occasionally Erythrophagocytosis.
   More tests-
   Presence of Schizonts in Peripheral blood film.
   Peripheral Leucocytosis.
   High serum tumour Necrosis factor alpha.
   Haematocrit (<20%)
   Hb < 7.1 g/dl
   Blood glucose < 2.2 mmol / litre
   Serum Creatinine >265 mumol / litre
   Raised Serum Aminotransferase.

   Cerebral Malaria may be a complication. It should be differentiated from Meningitis.

   New Techniques
   Staining of DNA and RNA by a fluorescent probe.

2) Anemia

- **Reduced Hb concentration**
- **Iron deficiency develops slowly**
- **Glossitis – Angular stomatitis**
- **Brittle nails and spoon- shaped**
- **(koilonychia)**

Causes – Dietary lack

Previous history of gastrectomy
Chronic blood loss in Menorrhagia
Gastro- intestinal Neoplasm
Peptic Ulcer

3) Deficiency of Vitamin B12

Anemia signs and symptoms
Mild Jaundice
Tongue may become red, smooth and sore and the patient may co plain of indigestion and diarrhoea
Subacute Combined degeneration of Spinal cord develops with damage to the cortico- spinal tracts and dorsal columns
Peripheral Neuropathy resulting in abnormal gain with Motor and Sensory loss.

Pernicious Anemia is the most common cause of Vitamin B12 deficiency.
It results in loss of Intrinsic factor, abnormal gait.

4) Hemolytic Anemia

Anemia due to premature destruction of RBC(Congenital or Acquired)

Causes –

Intrinsic abnormalities of cell membrane

(Congenital Spherocytosis)

Metabolism

Hemoglobin structure (sickle cell anemia)

Acquired hemolysis is usually of recent onset and results from cells being exposed to the destructive effects of antibodies, toxic chemicals, poison , or direct injury.

Spleen is often palpable

Severe long – standing hemolysis if Congenital origin.

Distortion if the bines of skull and face due to bine marrow hyoerplasia

(Radiological thickening of diploe)

Skin ulceration if lower legs.

Haemoglobinuria

Smoky urine

Increased urinary urobilinogen from the enterohepatic circulation and urine darkens on standing.

In hemolytic anaemia, no bilirubin appears in urine, because excess plasma bilirubin is unconjugated

5) Unconjugated hyperbilirubinemia
(Evidence of increased erythrocytes destruction)
Accompanied by increased uribilinogen excretion in urine.

Evidence specific to Intravascular haemolysis –
Haemoglobinemia
Haemoglobinuria

Haemolytic crisis
(causes enlargement of spleen during Infections and activation of reticuloendothelial system)
- **Heinz bodies (denatured Haemoglobin precipitates) in Peripheral red blood cells.**

6) Beta – Thalessemia major
(Cooley's anemia)
RBC destruction in bone marrow and spleen.
This causes anaemia and stimulates increased kidney erythropoetin production.
Deposition of iron in tissues (haemosiderosis) –
due to increased gastro – intestinal absorption of iron
Pancreatic haemosiderosis resulting in Diabetes.
Hepatic haemosiderosis resulting in Cirrhosis.
Cardiac haemosiderosis resulting in arrhythmias, heart blocks, Congestive Heart Failure.
Deposition of iron in various endocrine glands.

“Skull Radiograph “ shows “ hair on end “ appearance and generalized widening of medullary spaces.
Evidence that both parents have Thalassemia major.

7) Megaloblastic Macrocytic Anaemia

Megaloblasts are abnormal erythroblasts seen in bone marrow of patients with deficiency of Vitamin B12, folate or both.
Megaloblasts are abnormally large in size and nucleated. They are well haemoglobinised.
Vitamin B12 and folate are essential for DNA synthesis. Deficiency causes failure of DNA synthesis. This results in abnormal cell proliferation. Due to abnormality , these cells die and release Lactate Dehydrogenase (LDH)

8) Myelodysplstic Syndrome (MDS)

Subtype of Myeloid Neoplasms
Characterised by clonal proliferation of haemopoeitic cells , including erythroid, myeloid and megakaryocytic forms.
Leads to ineffective haemopoiesis and apoptosis.
And leads to cytopenia.
Signs –
Anaemia
Leucopenia
Thrombocytopenia

Extramedullary haematopoeisis leads to hepatomegaly and splenomegaly.

9) Hairy Cell Leukemia

Malignant expansion of beta lymphocytes .

Symptoms – Massive Splenomegaly, pancytopenia and vasculitis (erythema and cutaneous nodules)

Hip pain due to bone lesions.

Signs –

Pancytopenia

Peripheral smear shows characteristic hairy c cells

These are Malignant B Lymphocytes

Hairy structures on the surface of the cell are cytoplamic projections.

Eccentrically placed nucleus and foamy cytoplasm .

These hairy cells stain positively for Tartarate Resident Acid Phosphatase (TRAP)

Bone Marrow is difficult to be aspirated (“dry tap”) and biopsy shows Fibrosis with Infiltration by Mononuclear cells and hairy cells.

Splenic histology reveals Mononuclear cell infiltration of red pulp and engorgement of sinuses.

Liver biopsy reveals Infiltration of portal triads by hairy cells .

10) Kala Azar

Leishmania Donovani (parasite) causes Kala Azar (Parasite of Reticulo-endothelial system)

1. **Amastigote stage**

Aflagellar stage, present in the cells of Reticulo-endothelial system (macrophage , monocytes, polymorphonuclear leucocytes or endothelial cells) of Vertebrate host(man , dog etc)

2. **Promastigote stage**

Flagellar state in insect vectors (sandflies) and in culture.

Female sandfly is a blood sucking insect . It draws amastigote forms during blood meal and reach the mid- gut of the insect. Thry develop into promastigote firms which she regurgitates into healthy person while biting. They are engulfed by macrophages and converted to amastigotes.

Peripheral blood smear -
Has amastigote forms
Pancytopenia
Elevation of serum globulin level with reversal of albumin- globulin
Ratio.

Blood culture can demonstrate promastigote forms.

Bone Marrow Aspiration shows-
Amastigote forms

Splenic puncture shows-
Amastigote forms in stained films
Promastigote forms in culture.

ELISA
Detects the Leishmania Donovani antigen.

Latex Agglutination test
(KATEX)
For the detection of antigen in Urine Specimen.

LIVER FUNCTION TEST
For the biological function of hemoglobin to carry oxygen , the iron should remain in the ferrous state. Hemoglobin (Fe 2+) can be oxidized to methemoglobin (Fe3+)
In normal circumstances, the molecular oxygen does not oxidize Hb, it only loosely binds to form oxyhemoglobin.

Carbon monoxide (CO) is a toxic individual pollutant that can bind with Hb in the same manner as O2 binds.
CO has about 200 times more affinity than O2 for binding with Hb.
Abnormal hemoglobin are the resultant of mutations in the genes that code for alpha or beta chains of globin. As many as 400 mutant hemoglobins are known . About 95 % of them are due to alteration in a single amino acids of globin.
Sickle cell anaemia (HbS) and hemoglobin C disease (Hb C) are the classical examples of abnormal hemoglobins
Thalassemias are caused by decreased synthesis of normal hemoglobin.

Degradation of heme to bile pigments
Erythrocytes are removed from circulation after a lifespan of 120 days. They are taken up and degraded by the macrophages o reticulo- endothelial (RE) system in Spleen and Liver.

Globin – degraded to amino acids.
Heme – 80 % of heme is from erythrocytes
20 % from immarure RBC , myoglobin and cytichromes.

Macrophages in Liver take up erythrocytes and bilirubin is produced , which then firm bilirubin – albumen complex in blood and Bilirubin diglucoronide in Liver.

Urobilinogen breaks down to urobilin in kidney and is excreted in Urine.

And Microbial enzymes in Intestine breaks urobilinogen to Stercobilin and is excreted j faeces.

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As the albumen – bilirubin complex dissociation and is taken up by sinusoidal surface of the hepatocytes by a carrier mediated active transport.

In the Liver, bilirubin is conjugated with two molecules of glucoronate supplied by UDP- Glucoronate. The reaction catalyzed by UDP- Glucoronyltransferase results in the formation of a water soluble bilirubin diglucoronide. When bilirubin monogkucoronides also accumulate in the body.

Conjugated bilirubin is connected into bile canaliculi against the concentration gradient which then enters the bile . This transport is active and energy dependent and is easily susceptible to any impairment in the liver function.

Fate of bilirubin

Bilirubin glucoronides are hydrolyzed in the intestine by specific bacterial enzymes called beta – glucoronidases to liberate bilirubin. The latter is then converted to urobilinogen, small part of which may be reabsorbed in the circulation.

Urobilinogen can be converted to into urobilin (yellow colour compound) in kidney and excreted. This causes yellow colour in urine. (due to urobilin)

A major part of urobilinogen is converted by bacteria to stercobilin that is excreted in faeces. Brown colour of faeces is due to Stercobilin.

Normal serum bilirubin level is 0.3 to 1.0 mg/dl.

Unconjugated hyperbilirubinemia is characterized by serum level less than 6 mg /dl and absence of bilirubinuria . Conjugated hyperbilirubinemia is characterised by higher levels of bilirubin and bilirubinemia.

Fluctuating hyperbilirubinemia is seen in gall stones, carcinoma of Ampullaif Vater , chronic hepatitis, hemolytic anaemias.

Absence of bilirubin in urine in a jaundiced patient points to unconjugated hypefbilirubimemia .

In conjugated hyperbilirubinemia , urine contains bilirubin.

Enzymes

Aspartate Aminotransferase (AST, SGOT) and Alanine Aminotransferase (ALT, SGPT)

Normal value – 5 to 40 IU/L

Aminotransferase levels are useful to differentiate between Hepatocellular Jaundice and Obstructive Jaundice.

ALT is much higher in Liver than other tissues (kidney, heart muscle)

ALT is a sensitive index of hepatic damage.

AST exists as two different isoenzymes , Mitochondrial and Cytoplasmic form.
It is found in highest concentration in heart as compared to other tissues, such as Liver, Skeletal muscle and Kidney.

Alkaline Phosphatase (ALP)
Normal serum level is 3 to 13 KA units (80 to 240 IU/L)
Serum Alkaline Phophatase is elevated as it is released from the cells of damaged bile duct.

Serum Albumen is low in Cirrhosis and Chronic Hepatitis.

Ceruloplasmin
It is synthesized in liver and is an Acute phase reactant. It binds with Copper and serves as major carrier fir copper in the blood.
Normal plasma level of Ceruloplasmin is
20 to 60 mg/ dl
Levels are elevated in Infections , Rhematoid Arthritis and Liver diseases.
In Wilson's disease, Ceruloplasmin is depressed due to decreased rate of synthesis.

KIDNEY FUNCTION TEST

The composition of glomerular filtrate is modified by tubular reabsorption and secretion. Renal damage reduces functioning tubular mass.

1) **Urine volume**
2) **Urine turbidity**
3) **Proteinuria**
4) **Lipiduria**
5) **Hyper- protenemia**
6) **A:G (Albumen- Globulin) ratio**
7) **Creatinine**
8) **Serum urea**
9) **Glo erular Filtration Rate (GFR)**
10) **Haematuria**

Cystatin C is good indicator for GFR. The Protease inhibitor Cystatin C is a non- glycosylated low molecular weight protein. It is marker produced by all nucleated cells at constant rate and is filteted by Glomerul

Ketonuria

An abnormally high number of ketone bodies in the urine. Ketone bodies accumulate in urine when the body is using fat, rather than sugar, for energy.

Oliguria
Below normal urinary output.

Pneumaturia
The passage if gas in the urine.

Polyuria
Excessive urinary output

Proteinuria
Abnormal secretion of protein in urine.

Uremia
The excessive accumulation in the blood of the byproducts of protein metabolism , especially urea. This is a toxic condition.

Blood Urea Nitrogen

The level of urea in the blood is measured.
Urea is normally not present. Its presence in the blood can be a sign of kidney failure.

Creatinine Clearance Test

The amount of
Creatinine excreted in urine is measured ivera specific period of time, usually 24 hours.
Used to evaluate Renal functioning.

URINALYSIS

Bilirubin – A pigment that occurs when hemoglobin , a component of Red Blood Cell is broken down. Its presence may be a sign of an abnormal gall bladder or liver condition.

Glucose – indicates Diabetes Mellitus , a metabolic disorder in which the body cannot properly use carbohydrate.

pH – alkalinity or acidity of the urine.

Sediment - It indicates the presence of bacteria, blood cells, epithelial cells, or crystals.

Specific gravity - % of solids in urine as compared to liquid. In individuals with Diabetes Mellitus , the specific gravity will be higher than normal because of presence of sugar in urine.

Potassium - in diarrhoea and dietary deficiency

Sodium – usually diagnoses the following condition : calcium stones, anti- hypertensive, Acute kidney injury.

Urine Culture – For accurate diagnosis of UTI(Urinary Tract Infection)